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VACCINATION AGAINST INFLUENZA *

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SYNOPSIS

This paper reviews studies which have been carried out during the past twenty years in the United States of America to investigate the suitability of various vaccines and vaccination methods for immunizing man against the different influenza virus strains. A number of investigations in closed communities, such as children's institutions, army and navy units, and medical schools, are described. The author discusses the comparative value of the techniques employed in preparing vaccines, and the use of adjuvants in improving the response.

1935-41

Active virus

The initial studies on vaccination of human subjects with influenza virus were undertaken to determine whether the administration to man of active virus by the subcutaneous or intracutaneous routes would incite infection, and in order to gain evidence of the degree of antibody response which could be elicited. Utilizing the PR8 strain of type A virus it was found that virus propagated in chick embryo—Tyrode's culture, given by either route, did result in the production of neutralizing antibodies to titres comparable with those observed in convalescent patients (Francis & Magill [10, 11]). The peak was reached in the second week and a relatively high level persisted during the observation period of six months. There was little evidence that additional doses after the peak was reached had any significant influence in adults. Even at that time the suggestion was made that those with the higher titres initially did not exhibit as great increments as those with lower titres.

Stokes, Chenoweth, Waltz, Gladen & Shaw [57] prepared Berkefeld filtrates of swine virus and of human virus A from 10% mouse-lung suspensions, and then inoculated members of a children's institution with three doses intramuscularly, while retaining as controls an uninoculated

* Many of the studies reported in this paper were conducted by the Commission on Influenza of the US Armed Forces Epidemiological Board, and were supported by the Office of the Surgeon General, Department of the Army, Washington, D.C., USA.

group approximately twice as large. During an outbreak of respiratory disease in February 1936, they recorded an incidence of 12.5% and 12.4% of febrile illness in the controls and in the groups vaccinated with swine virus, respectively, but only 2.7% in those receiving the PR8 vaccine, although only 31% of this latter group showed an increase of antibodies after vaccination. The nature of the disease was not clearly established, but it did occur in a season in which influenza B was subsequently shown to have been very prevalent. Nevertheless, certain serological tests suggested that some cases of influenza A were occurring. The incidence of afebrile disease was uninfluenced.

The following year (see Stokes, McGuinness, Langner & Shaw [58]) an expanded study in similar institutions was carried out with culture virus of the PR8 strain. In this instance some strains of influenza virus A were recovered from patients with influenza and a reduction in febrile illness indicated that vaccination with active virus had been influential in reducing the incidence of influenza in children, and it was suggested that some of the differences in effect observed in the various institutions might be related to the length of interval between inoculation and appearance of disease.

Inactivated virus

In the same winter Smith, Andrewes & Stuart-Harris [56] attempted a controlled prophylactic study, among military forces in England, of subcutaneous vaccination with filtrates of a 10% suspension of the WS strain from mouse lung, inactivated by 1 : 2,000 formalin. The disease occurred before vaccination was fully carried out, and the incidence was low. No effect of the vaccination was noted. A polyvalent vaccine similarly prepared was employed in 1938-9, but no evidence of protection was observed in the group under study (Stuart-Harris and co-workers [59, 60]). Taylor & Dreguss [61] observed no significant effect in their study of vaccinated and unvaccinated individuals, and attention was drawn to the fact that the epidemic strain differed from the WS strain of the vaccine.

The next extensive studies were those of Horsfall, Lennette, Rickard & Hirst [30] with material prepared from chick-embryo tissue previously inoculated with PR8 strain of A virus and a strain of canine distemper virus. The minced embryo suspension was inactivated with 1 : 4,400 formaldehyde, and 1.0-ml doses were given subcutaneously to individuals in a number of institutions. From 30% to 60% of the populations, totalling some 16,000, were vaccinated; the others served as controls. Although a difference in total incidence between vaccinated and controls was observed during an epidemic period, there was a significant reduction in only two of ten vaccinated groups; in two other groups the incidence was higher among the vaccinated than among the controls. The ineffectiveness was attributed, in part, to the lack of potency of one large batch of the vaccine. Brown

et al.,[1] using a similar preparation, reported an inconstant but final reduction in incidence from 25% in the controls to 13% in the vaccinated groups. Dalldorf, Whitney & Ruskin,[2] in a limited study of the same material, noted no difference. Siegel et al.[52] employed different vaccine preparations through three successive outbreaks of influenza A in 1937, 1939, and 1941, but observed no difference between vaccinated and unvaccinated groups.

Conclusions

At this stage, then, there was little consistent evidence from field trials that subcutaneous vaccination afforded effective protection against influenza in times of epidemic, even though studies had adequately shown that vaccination with various materials could induce an increase in antibody titre comparable to that observed after infection. The persistence of satisfactory levels of antibody for a period of several months was the rule, and continued emphasis was placed upon an apparent correlation between the height of the level of antibodies and resistance to the disease. The only conclusions which could be drawn, therefore, were that if influenza was not prevented in the vaccinated individuals it was because (*a*) the vaccine was not of sufficient potency to excite antibody levels to uniformly adequate heights; (*b*) the strains in the vaccine were not sufficiently similar to those causing the epidemic; or (*c*) the level of antibodies in the blood, resulting from vaccination, was not sufficiently high to control influenza.

There were obvious reasons for suggesting that the materials employed in the vaccination studies were not particularly potent in virus content. Eaton & Martin[4] noted that the titres obtained with the complex influenza-distemper vaccine were not as high as those in convalescent patients.

Quantitative determinations of antibody levels, in an attempt to appraise the nature of clinical illness occurring in the study groups, had not been followed extensively until the investigations of Rickard, Horsfall, Hirst & Lennette.[40] These were difficult to interpret because a large number of individuals with high titres who showed no increase in convalescence were classified as having a different disease, influenza Y. This conclusion was dependent to some extent upon Horsfall & Rickard's [31] conclusion that the serological response of the patient convalescent from influenza A was uniform against all strains of type A. Hence, if no increase was observed the causative agent must be of another type.

Effect of concentration on response to active virus

It had been demonstrated in mice vaccinated intraperitoneally with active virus that there was a progressive increase in the active immunity obtained as the amount of virus in the inoculum increased up to the point

where maximal infective doses were resisted (Francis [7]). Subsequent to these vaccination studies in man, Hirst, Rickard, Whitman & Horsfall [28] carried out a study comparing the effects of different amounts of active and inactive virus obtained from infected chick embryo and from allantoic fluid. The two most concentrated vaccines were prepared by high-speed centrifugation. Using adequate numbers of human subjects, and measuring their antibody levels by the Hirst antihaemagglutination technique, they found with active influenza virus that the antibody titres attained increased as the amount of virus in the inoculum increased. As shown in the following tabulation, however, the increase was not in direct proportion.

Vaccine 56 contained the equivalent of	0.05	ml of PR8 allantoic fluid
Vaccine 55 contained the equivalent of	0.5	ml of PR8 allantoic fluid
Vaccine 57 contained the equivalent of	5.0	ml of PR8 allantoic fluid
Vaccine 64 contained the equivalent of	24.0	ml of PR8 allantoic fluid
	+ 10.0	ml of WS allantoic fluid

An increase of ten times the dose as between vaccines 56 and 55 gave no more than a 20% increase in titre, and to obtain levels in the same range as those observed in patients convalescent from influenza A—i.e., twice as high as those reached with unconcentrated fluid—ten times as much virus (vaccine 57) was required as was present in unconcentrated allantoic fluid (vaccine 55). A sixfold increase in the amount of virus administered beyond that quantity resulted in no significant enhancement of the titre. These results clearly indicated that unconcentrated, infected allantoic fluid definitely stimulated antibody production, but that there was a sharp increase in the effectiveness as a concentration approximately ten times greater was approached, beyond which there was little additional gain. In the case of B virus, a concentration only four times greater than that of unconcentrated allantoic fluid was required. One other feature of interest was that the titres fell rapidly during the six weeks after vaccination—a finding at variance with other studies. One can ask whether the process of concentration itself influenced the antigenic potency by removal of stabilizing substances. Addition of the distemper virus had no influence on the increase in titre.

Effect of inactivation

When unconcentrated allantoic fluid was inactivated, the antigenicity in terms of antihaemagglutination was not influenced.

In contrast, a series of preparations of formalinized allantoic fluid tested in 1941 showed sharp deterioration of immunizing potency for mice, even though antibody was still elicited in man.[12] Inactivation of mouse-lung virus has usually resulted in a definite decline of immunizing potency to about one-tenth the original, even with mild agents such as

ultra-violet light or soaps. Smith, Andrewes & Laidlaw [55] had stated that certain formalinized preparations appeared to be as effective as active ones, but they were not thoroughly titrated.

In 1942, Hirst, Rickard & Whitman [27] and Hare, McClelland & Morgan [21] demonstrated that virus could be concentrated from allantoic fluid by freezing and then thawing at a low temperature. In the latter state, virus precipitated and separated from other constituents of the fluid. Both groups of investigators (Hirst et al.;[27] Hare, Morgan, Jackson & Stamatis [22]) demonstrated that after inactivation a concentrated vaccine induced better antibody responses than had been obtained with unconcentrated vaccine and as good, at least, as those obtained with active centrifuged material.

1942-5: Concentrated and Inactivated A and B Vaccines

The Commission on Influenza of the US Armed Forces Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army proceeded at this stage to undertake studies, in 1942-3, of the effect of concentrated vaccine containing influenza viruses of types A and B, inactivated with formalin. The studies were devised so that alternate persons would receive vaccine of a control inoculation. The records of vaccination were kept separate, so as to avoid reference to them until the study was completed. Determinations of the antibody levels induced by the vaccine were made, and close, continued clinical observation was kept of the illnesses in the experimental groups. In addition, material for virus study and for serological determinations was obtained from cases of respiratory disease.

For the study at Cornell University Medical College, N.Y., allantoic vaccine concentrated by freezing was employed. (Difficulties in the preparation were presented by the problem of rendering and maintaining large amounts bacteriologically sterile.)

For the study in Michigan a process was employed which takes advantage of the adsorption of influenza virus onto the erythrocytes of the chick. McClelland & Hare [33] in their original paper suggested this as a method of concentration, since the virus could be detected as adsorbed onto the red blood cells. By this means, the major portion of the virus can be removed by the erythrocytes from the allantoic fluid while most of the normal protein remains in the fluid. Hirst [25] had shown that the virus readily eluted from the erythrocytes at or above room temperature. The agglutination is carried out in the cold and the supernatant fluid is removed; to the mass of agglutinated red cells is added one-tenth of the original volume of physiological saline and the material is then kept at 23°-37°C for from one to two hours, during which time virus elutes from the red cells (Francis & Salk [13]). The vaccine is then constituted so that

1.0 ml contains the PR8, type A, strain and the Lee, type B, strain, each obtained from 5.0 ml of the allantoic fluid. Virus is inactivated by formalin 1:2,000, and a mild bacteriostatic is added.

Because of the preceding two-year cycle of influenza A observed in the USA, the winter of 1942 was expected to bring an outbreak of that disease. It did not occur. Late in the season—in March 1943—a mild, unsuspected prevalence of influenza B was detected, largely by serological tests. No influenza A appeared in the group of approximately 8,000 persons studied. It had been possible, however, to demonstrate that excellent antibody responses to both types of virus had occurred, and that in four months a decline of about one-third from the peak titre had taken place (Salk, Pearson, Brown, Smyth & Francis [49]).

Since it had been found, however, that infection could be induced experimentally without serious risk, it seemed desirable to test the efficacy of vaccination in this way. Henle, Henle & Stokes [23] had reported that a mixture of allantoic fluids containing PR8, WS, and Mel strains of type A virus inactivated by formalin, given four months earlier, or inactivated allantoic fluid containing PR8 alone given two-and-a-half weeks earlier, protected all but one of 44 children against inhalation of a recently isolated strain cultivated in eggs (F-99). Ten out of 28 controls became ill.

On the other hand, our studies of resistance to B influenza virus had shown that four months after being sprayed with this virus in allantoic fluid a majority of the people thus infected had symptoms when sprayed again with the same virus, and one-third had just as severe illness the second time, even though their antibody levels were markedly higher than the original titres. On the other hand, a group of 66 vaccinated subjects was selected and sprayed with a strain of type A virus relatively closely related antigenically, but not identical with that used in the vaccine. Of 36 unvaccinated controls, half developed fever of 100°F (37.8°C) or more and symptoms of influenza; of 28 vaccinated four-and-a-half months before, 32% had similar experience; among the 38 vaccinated two weeks before testing, 6, or 16%, had fevers of 100°F—but none higher—in sharp contrast to the other groups (Francis, Salk, Pearson & Brown [15, 16]).

In a group of 96 tested by inhalation of test B virus (Salk, Pearson, Brown & Francis [47, 48]) 11, or 41%, of 27 unvaccinated individuals became ill; of the 79 vaccinated either four-and-a-half months or four weeks—or at both times—before testing, only 8, or 10%, had fevers of 100°F (37.8°C) and none reached 101°F (38.3°C). These results indicated a stronger effect of subcutaneous vaccination than was observed against influenza A, although the B test may have been somewhat less severe. In comparison with the resistance exhibited four months after actual intranasal infection, the effect of subcutaneous vaccination was much more impressive.

In 1943 a more extensive investigation was made by the Commission on Influenza in a series of army units in colleges throughout the USA,

with vaccine prepared by adsorption and elution but incorporating equal parts of PR8 and Weiss strains as the A component and Lee strain as B component. The study comprised six different groups of investigators working in nine different universities, and an effort was made to maintain comparable conditions throughout. In all but one area, alternate men of each unit served as vaccinated and controls, the latter being inoculated with physiological salt solution to which formalin (1 : 2,000) and phenylmercuric nitrate (1 : 100,000) had been added ; the same lots of vaccine were employed throughout; the same basic plan of clinical observation and handling, and of etiological studies, was maintained. [5, 8, 20, 26, 34, 41, 46, 62] There were 6,263 vaccinated individuals and 6,211 inoculated controls. Most of the vaccination was done in late October and early November, and was soon put to the test by an epidemic of A influenza. In the total group an incidence of 2.2% of hospitalized cases was observed among the vaccinated persons and 7.1% in the control group, with a consistent and significant reduction in all but the one study in California, where a number of deviations in the pattern of the study occurred, and where Eaton & Meiklejohn [5] attributed the lack of effect to the occurrence of an antigenically divergent strain of virus. In five of the nine units the incidence in the controls was between three-and-a-half and six times as great as in the vaccinated—an effect which may be considered minimal since the frequency of illness among those who had been neither vaccinated nor inoculated was greater than that among controls of the vaccinated groups, thus suggesting a reduction in risk among the controls through the reduction of susceptibility in the group as a whole, owing to the presence of vaccinated individuals.

The results clearly demonstrated that a consistent and pronounced lowering in the incidence of clinical influenza A was attained by subcutaneous vaccination with inactive influenza virus.

Since in two locations (Hale & McKee; [20] Hirst, Plummer & Friedewald [26]) the epidemic began at about the time of vaccination, the curves of incidence of disease in vaccinated persons and controls could be followed. In the first week no differences were observed, but after six or seven days the curves diverged sharply as the incidence in the vaccinated group decreased; this indicated that the prophylactic effect of vaccine began at a time when circulating antibodies are ordinarily beginning to rise.

In 1945, by virtue of the uniform vaccination of the entire personnel of the US army, and the occurrence of an epidemic of influenza B, it was possible through the Commission on Influenza to gain information about the effect of the same type of vaccine against that disease. At the University of Michigan there were 1,100 men in the unvaccinated naval unit, and 600 in the army unit, all of whom were vaccinated. The units lived under similar conditions and were under the medical supervision of the same personnel. During the ensuing epidemic of influenza B, 109 cases, an incidence of 9.9%, occurred in the unvaccinated group and only seven cases, or 1.2%, in the

vaccinated (Francis, Salk & Brace [14]) (see table I). At Yale University, with similar circumstances and numbers, there were three cases, or 0.5%, among 550 vaccinated army personnel, and 132, or 12.5%, among 1,050 unvaccinated naval students (Hirst, Vilches, Rogers & Robbins [29]).

TABLE I. RESULTS OF VACCINATION AGAINST INFLUENZA IN SIX COMMUNITIES

Location	Vaccinated			Unvaccinated		
	number	cases found	incidence (%)	number	cases found	incidence (%)
Michigan, Mich. . .	600	7	1.2	1,100	109	9.9
Yale, Conn.	550	3	0.5	1,050	132	12.5
Alabama, Ala. . .	30	2	6.7	95	18	18.9
Washington, D.C. .	360	7	1.9	4,280	352	8.2
Glasgow, Scotland	115	2	1.7	105	9	8.6
Woolwich, England	609	31	5.1	622	68	10.9

Although these investigations did not employ alternate controls within the same units, the groups were so similar in all other respects as to make them readily comparable. The difference in incidence in the two groups certainly appears to be the effect of vaccination. Further support for this conclusion is found in the fact that the vaccinated army units in the same geographical areas had a sharply lower incidence of influenza during the epidemic period than did naval personnel. The fact that a stronger effect was observed against influenza B than in the earlier studies against influenza A is in keeping with the results noted with experimental infection after vaccination, and also with the readier immunizing effect of B virus in mice. In addition, the fact that all members of the one group of units were vaccinated and all those in the control units were unvaccinated may have helped to enhance differences in the mass resistance of the two groups. This influence was exhibited despite the fact that distinct differences could be demonstrated in the serological character of the epidemic strains from that of the Lee strain in the vaccine.

A small group at the University of Alabama also showed a reduced incidence in the vaccinated individuals (Friedman [19]). Norwood & Sachs [38] observed a sharp reduction in an industrial plant in Washington. Two groups were studied by Dudgeon, Stuart-Harris, Andrewes, Glover & Bradley [3] with vaccine of the same character as that used in the USA. The incidence of influenza B was low, and the inoculations were not undertaken until the outbreak was under way. Nevertheless, the results at both Glasgow and Woolwich tended to be in favour of the vaccine.

1946-53

Problem of strain characteristics

After a lapse of three years from the influenza A epidemic of 1943, an outbreak of the disease was anticipated in the winter of 1946-7. At the end of October 1946, a vaccination study was again instituted at the University of Michigan, where 10,328 persons received eluate vaccine containing the same strains as in previous years; 7,615 were unvaccinated. When influenza occurred during March 1947, no evidence of protective effect was demonstrated. The incidence in vaccinated subjects was 7.19% and in controls 8.09% (Francis, Salk & Quilligan[17]). Although the outbreak did not begin until four months after vaccination, the evidence was clear that the antibody titres of the vaccinated individuals, when measured against the vaccine strains, remained at about the same level as those observed two weeks after vaccination. On the other hand, the titres of the vaccinated persons were no higher than those of the controls when tested against epidemic strains.

Further evidence of the inefficacy of the vaccine was the high frequency of the disease observed in vaccinated groups, even though comparable numbers of controls were not available (Sigel et al.[53]). Data from the US army as a whole yielded no evidence of efficacy.[51] In a controlled study, Fowle & Weightman[6] noted incidences of 7.05% in 1,250 vaccinated individuals, and 7.3% in 794 unvaccinated persons. Loosli, Schoenberger & Barnett[32] observed the same incidence, 9.5%, in 790 vaccinated and 1,230 unvaccinated individuals, in a test of three different preparations of vaccine. Van Ravenswaay[63] observed 20.2% incidence in 237 vaccinated and 27.8% in 284 unvaccinated persons.

The British studies[37] involved a variety of institutional and military groups totalling 20,000 persons. The vaccine was prepared by red blood cell adsorption and elution with either the Mel or the PR8 strain of A virus. The incidence of influenza was low; infection was absent in many of the units, and the actual identification of influenza was lacking in others. In two schools a mild reduction of incidence was noted: in one, from 22% among controls to 11% in vaccinated individuals, and in the other from 17.3% to 11%. Otherwise, no differences were observed.

The absence of prophylactic effect was so clear-cut as to differ sharply from, and enhance the significance of, the results of 1943 and 1945. Studies from numerous laboratories clearly showed the serological difference of the epidemic strains from the PR8 and Weiss type A strains incorporated in the vaccine.[17, 32, 39, 53, 54] Antibodies to the epidemic strains were not generally induced by vaccination, or occurred only to low levels, although excellent responses to the vaccine strains were demon-

strable. There was no significant difference in mean titres to the 1947 strains among vaccinated and unvaccinated persons in the acute stage of the disease, and the antibody increase observed in convalescence was essentially the same in the two groups (Francis, Salk & Quilligan [17]). Furthermore, many sera from the 1943 epidemic, which showed a marked rise to the PR8 strain, failed to show an antibody increase to the 1947 strains. That the strains were of type A was shown by the fact that the majority of convalescent patients exhibited an antibody rise to PR8 or to other A strains; this was demonstrable by neutralization, haemagglutination-inhibition, or complement-fixation tests. That vaccinated individuals showed less rise to the PR8 than to 1947 strains after infection is to be expected because of their high post-vaccination titres to that strain.

The experience of 1947 clearly established an affirmative answer to one question which had been constantly present. Can strain differences demonstrable serologically be of significance in immunization ? In order to meet the antigenic variant, the Commission on Influenza recommended that a representative of the 1947 strains, which were designated A-prime, be incorporated in the subsequent vaccines.

Since that time the studies of the Commission have continued in US military installations, with various preparations of vaccine designed to give further information. The major studies are those conducted each year at Fort Dix, New Jersey, and at Fort Ord, California, both in recruit populations.

In the winter of 1947-8, the first of these studies, by Salk & Suriano [50] at Fort Dix, was concerned with comparing the effect of an "old-formula" vaccine containing PR8, Weiss, and Lee strains, prepared by adsorption and elution, with a vaccine containing PR8, FM1 (1947), and Lee, prepared by Sharples centrifugation. The second vaccine induced much better antibody titres to the A-prime strain while the first, although a year old at the time of use, induced somewhat better responses to the PR8 and Lee strains. There was a slight prevalence of A-prime influenza during the period of observation, and while the bulk of respiratory disease in the population was non-influenzal, a significant reduction in the number of cases was demonstrable in the group receiving vaccine containing the FM1 strain.

In 1948-9 the incidence of influenza was so small as to furnish little information about the effect of vaccination upon the disease. However, serological results obtained with monovalent vaccines in that and in the succeeding year have been reported.[38] It was readily demonstrated by these means that in man the FM1 vaccine stimulated antibody rises to the PR8 strain of nearly the same magnitude as to itself, although the reciprocal with PR8 vaccine was lacking, as shown in 1947. These data provided a beginning for further interpretation and understanding of strain differences.

The following year, 1949-50, influenza caused by A-prime virus was more prevalent. The studies at Fort Ord again tested monovalent vaccines, PR8 (A), FM1 (A-prime), and Lee (B), together with control saline inoculations.[35] Equal numbers of each unit received one of the four preparations.

The incidence in the four training groups is shown in the following tabulation :

Vaccine	*Number vaccinated*	*Number of cases*	*Incidence (%)*
FM1 strain	528	5	0.9
PR8 strain	553	21	3.8
Lee strain	536	23	4.3
Control	534	25	4.7

The identification of cases was based upon complement-fixation and haemagglutination-inhibition tests. No difference in the incidence of non-influenzal respiratory disease was noted between the groups. Thus, despite the low incidence, careful examination revealed that FM1 vaccine had been effective against the current A-prime strain which exhibited a measurable serological difference from that of the vaccine.

In 1950-1, in the same installations, two vaccines and saline control were tested. The A vaccine contained equal parts of the PR8, FM1 (1947 A-prime), and Cuppett (1950 A-prime) strains at a level of 500 chick-cell agglutinating (CCA) units, 200 higher than in the previous three years. The Lee strain represented the B vaccine at 500 CCA units. Results of this year have not been published but, at Fort Dix, Dr. J. E. Salk and Dr. E. Lennette (personal communication) observed a 4:1 difference in favour of the vaccinated individuals, and at Fort Ord similar results were noted.

Information from the present year (1953) with still more potent vaccine containing only A-prime strains is incomplete, but preliminary data again indicate a great advantage for the specifically vaccinated group, although the epidemic strain differs serologically from those in the vaccine.

The accumulated evidence with epidemics caused by A-prime strains, then, has been uniform in establishing the fact that influenza vaccine containing strains of that group continues to be effective. It also demonstrates that protection can be obtained by the use of vaccine strains which are not identical with those prevalent. This is important with regard to both cross immunity and antigenic composition.

In 1951-2, influenza B was prevalent. Once more, the data are incomplete, but, where the incidence was sufficient to permit measurement, the influence of vaccine was apparent. For example, at a children's institution equal numbers within each cottage received polyvalent A vaccine, B vaccine (Lee, 700 CCA units), or saline.[24] Although serological differences between the epidemic strain and that in the vaccine were clearly demonstrable, the

incidence in the B-vaccinated group, as shown in the following tabulation, was about one-third that in the other two.

Treatment	*Number treated*	*Cases*	*Incidence (%)*
B vaccine	207	15	7.2
A vaccine	218	39	17.9
Saline	212	44	20.8

The outbreak was essentially pure influenza B. It is of interest that the children in the B vaccinated group who developed the disease were the youngest children, and their antibody titres had shown a sharp decline from the immediate post-vaccination titres. This rapid decline in antibody level in three months has not been commonly observed in other studies; it appears to be a factor of age.

Use of adjuvants

In addition to the problem of strain characteristics, the concentration of virus antigen in vaccine is an important factor. In order to obtain high antibody levels, the amount of virus must be maintained well above the minimal level. In the earlier materials prepared by adsorption and elution the amount was essentially that derived from 10 ml of allantoic fluid. Later, an arbitrary level of 300 CCA units per ml was set, and the antibody responses were less marked. Subsequently, the concentrations have been increased to levels of 700-750 CCA units per ml and good titres have resulted. There is, however, the fact that some strains, especially the A-prime group, are less effective even in these amounts, owing to either an inherent antigenic defect or a lesser stability of the inactivated material.

A number of earlier studies had suggested the possibility of using lipids to enhance the effect but, because of serious accompanying reactions, the materials were not acceptable for human use. Recently, Salk and his associates have conducted extensive investigations of the use of virus first emulsified in Arlacel A (mannide mono-oleate) [a] and then suspended in a light mineral oil, a combination found by Freund and his associates [18] to eliminate the unfavourable local abscess production or extensive encephalomyelitic disturbances encountered with certain other preparations.

The studies have developed in a progressive manner, from observations of the responses in experimental animals receiving various combinations of virus and adjuvant to the testing in man of the efficiency and practicability of selected preparations. It was shown first in mice and monkeys that extremely high titres, in the thousands, of haemagglutination inhibitor or neutralizing antibody (in ovo) could be obtained with mixtures of adjuvant and quantities of virus which, in aqueous form, resulted in titres of approxi-

[a] Obtained from the Atlas Powder Co., Wilmington, Del., USA

mately 128. In monkeys the titres continued to rise progressively from levels of 256 in one week to a peak of 16,000 or more in eight weeks. After four months some decline in titre was commonly observed, but when moderate amounts of virus are considered the levels remain in the thousands at the end of a year. Moreover, an amount of virus which in the usual vaccine had little antigenic effect could, in conjunction with adjuvant, still elicit high titres (Salk, Laurent & Bailey; [45] Salk & Laurent [44]).

The extended studies in man have been presented in two publications (Salk, Bailey & Laurent; [43] Salk [42]). Basically, they demonstrate effects in man paralleling those observed in monkeys. The titres obtained with adjuvant vaccines greatly exceed those obtained with similar amounts of virus in aqueous vaccine. Antibody response to a polyvalent adjuvant vaccine containing 100 CCA units of each of three virus strains reached peak titres of approximately 2,000 in four months, declined in one year to levels of 512-1,024, and remained at around these levels during an additional year of observation. In comparison, persons receiving a preparation of aqueous vaccine containing 133 units of each strain had levels of approximately 256, which, with a slight decline, persisted thereafter at half the six-week level. In general, the maximum response to aqueous preparations is reached in two weeks in adults, and at this time usually significantly exceeds the mean titres obtained with adjuvant material. It has been clearly shown, however, that, in the presence of adjuvant, amounts of virus in the range of 10 CCA units can result in levels of antibody well above those following aqueous vaccine containing ten times as much virus. This readily offers opportunity for incorporating a number of strains differing antigenically, whereas the quantities required make this more difficult in polyvalent aqueous preparations. In this connexion, the data indicate that, with the abundant responses obtained, there is a greater capacity to overcome differences in antigenic structure of various strains. It may be pointed out, too, that the contingency of incorporating a new strain immediately into vaccine may well be met by the relatively little attention which need be given to high titres required for production of aqueous material.

The only untoward reactions observed to date occurred in a group of persons given vaccine prepared with a certain lot of Arlacel A. These reactions took the form of cyst-like accumulations which developed at the site of inoculation, in about 1% of the subjects, two to four months later. Although many possibilities were considered, it eventually appeared that the cause was impurities or unnecessary substances in that particular lot of emulsifier. These have been removed without altering the efficiency of the material, and later preparations have not exhibited this reactivity. In the meantime, tests have been devised for determining the presence of such harmful materials in experimental animals.

The possibility of carcinogenic effect has also been explored, and the resultant data indicate that the materials employed do not possess the

characteristics associated with the carcinogenic action of oils. Similarly, the risk of sensitization appears to be minimal.

The efficacy of adjuvant vaccine in protecting man against influenza has not yet been established, but if antibody levels are the deciding factor the evidence weighs heavily in its favour. The cost of its production is also considerably less than of aqueous vaccine. As with other prophylactic materials, additional data are needed as to the best and most stable strains for use in stimulating antibodies, and for giving wide coverage; accurate knowledge of ideal proportions, and exploration of other materials which may be of still further advantage as adjuvants would also be of great value.

Vaccination against influenza has been shown to be uniformly effective under a variety of conditions, when vaccines of proper constitution and potency are employed. Although the writer has consistently held that the epidemiological and serological data have indicated the probability that strain differences are important in the recurrences of influenza, he has also emphasized that the degree of variation so far observed is a limited one, representing more a rearrangement of viral components than complete loss or gain of basic constituents.[9] This is in disagreement with the thesis that the old antigens progressively disappear and are replaced by new, unrelated ones. Current analyses in our laboratory strongly support the concept that the antigenic constitution of influenza virus of a particular type can be mapped. It is the expectation, then, that vaccine can be so composed as to contain the different antigenic components required and will induce, as desired, the broad resistance which otherwise is acquired only by repeated exposures to the disease. There remains the problem of virulence, but most of the data indicate that even highly virulent strains can be counteracted by adequate vaccination, or by infection with mild strains. For example, mice infected with unadapted egg-passage lines become resistant to the highly virulent mouse line of the strain concerned. Moreover, relatively new antigenic strains are not necessarily highly virulent, as evidenced by the mild character of A-prime strains in 1946-7 and later. It is believed that the severity of influenza in the autumn of 1918 was the result of enhanced virulence through adaptation of a strain which had been in circulation and, certainly, was related antigenically to known strains of influenza virus. The fatality with which it was associated clearly seems to be related to physiological factors in the human host, as well as to the immunological factors which determined incidence. The latter can now be controlled.

In conclusion, the outlook for increasingly broad and effective prophylactic immunization against the range of influenza viruses is extremely promising. The studies from which present knowledge has developed represent a continued investigation of a complex field, moving with the accumulating evidence towards better understanding and practical prevention of the disease.

RÉSUMÉ

Les premières études sur la vaccination antigrippale eurent pour objet de déterminer si l'injection de virus actif par voie sous-cutanée ou intracutanée déclenchait l'infection et quel niveau d'anticorps pouvait être ainsi obtenu. De 1935-42, divers essais furent effectués dans des collectivités, avec le virus actif ou le virus inactivé par la formaline. Les résultats, inconstants, n'apportèrent pas de preuve évidente du succès de la vaccination antigrippale, bien que l'on ait observé, en maintes occasions, une augmentation de la teneur du sérum en anticorps. Cet échec pouvait être dû à l'activité insuffisante du vaccin, à des différences antigéniques entre la souche responsable de l'épidémie et la souche de virus employée comme vaccin, ou encore au fait que le titre des anticorps suscités par la vaccination était trop faible. Des expériences furent alors entreprises avec du virus concentré par congélation et décongélation, méthode qui permet d'éviter l'altération du pouvoir antigénique par la centrifugation. Dès 1942, la Commission on Influenza of the US Armed Forces Epidemiological Board entreprit des essais, dans diverses institutions et dans l'armée, avec des vaccins concentrés et inactivés, contenant les types A et B (souches PR8, Weiss ou Mel et Lee). Les résultats furent en général satisfaisants.

En 1946-47, cependant, la vaccination fut nettement moins efficace. Les recherches montrèrent qu'une nouvelle souche pathogène était en jeu, vis-à-vis de laquelle les souches composant le vaccin utilisé au cours des années précédentes étaient peu — ou pas — actives. Aussi la Commission recommanda-t-elle qu'une souche isolée en 1947 (FM1), variante antigénique du type A (désignée par A-prime) soit incorporée au vaccin. Depuis lors, les résultats de la vaccination ont été beaucoup plus favorables. Le vaccin FM1 se montra actif même contre une souche A-prime différente de la souche vaccinale, fait qui se confirma au cours des années. Le vaccin contenant la souche FM1 est encore efficace en 1953, bien que la souche causant l'épidémie n'ait pas été toujours identique à la souche vaccinale.

Pour obtenir une teneur suffisante en anticorps, il faut que la concentration du virus soit adéquate. D'une quantité de 10 ml de liquide allantoïque, utilisée au début, on passa à 300 puis à 700 et 750 unités CCA (chick-cell agglutinating). Il faut remarquer que certaines souches du sous-groupe A-prime sont moins actives à cette concentration élevée. On a proposé d'ajouter au virus diverses substances, pour augmenter son activité. L'émulsion du virus dans l'Arlacel A, puis sa mise en suspension dans une huile minérale légère, a donné de bons résultats. Chez les souris, les vaccins avec adjuvants ont décuplé la teneur en anticorps antihémagglutinants et neutralisants. Des teneurs élevées se sont maintenues dans le sang pendant une année, durée de l'expérience. Des quantités de virus qui, sous forme de vaccin ordinaire, n'ont que peu d'effet, deviennent très actives par addition d'adjuvants. Il en est de même des vaccins polyvalents, composés de plusieurs types et souches de virus. Il est possible, grâce aux adjuvants, de préparer des vaccins polyvalents efficaces, contenant plusieurs types d'antigènes, chacun sous un faible volume; les préparations aqueuses de tels vaccins sont difficilement applicables, en raison du volume considérable de virus qui est nécessaire. Les risques de cancérisation ou de sensibilisation par les adjuvants paraissent nuls.

La valeur protectrice pour l'homme des vaccins avec adjuvants n'a pas encore été établie, mais les résultats sur l'animal sont prometteurs, s'il s'avère que le niveau d'anticorps produit est un indice réel de l'immunité.

En conclusion, la vaccination antigrippale, effectuée au moyen de vaccins judicieusement composés et assez actifs s'est montrée efficace dans diverses conditions. L'auteur estime que les variations antigéniques, au sein d'un même type de virus, sont limitées

et indiquent un réarrangement des caractères antigéniques plutôt que la perte de certains d'entre eux et l'acquisition de nouveaux. La constitution antigénique d'un virus peut être exactement définie. La préparation de vaccins contenant différents composants antigéniques utiles permettra, à l'avenir, de provoquer une résistance étendue, qui ne peut être acquise actuellement que par de multiples expositions à l'infection.

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